Effect of lab procedures and ultrasonic bath cleaning on the pollution of customized implant abutments: an vitro study

Deborah ROTH, DDS, MS. Adjunct Head of clinic, Department of Removable and Implant Prosthodontics, CHU of Liège, Belgium. Dental Biomaterial research Unit, University of Liège, Belgium.

Dental Biomaterial research Unit, University of Liege, Belgium.

Marc LAMY, DDS, MS, PhD. Professor and Head, Department of Removable and Implant Prosthodontics, CHU of Liège, University of Liège, Belgium. Dental Biomaterial research Unit, University of Liège, Belgium. Dental Biomaterial research Unit, University of Liege, Belgium.

Philippe COMPERE, PhD, Adjunct Director. Laboratory of Functional and Evolutive Morphology, UR-FOCUS, Biomaterials Interfaculty Center (CEIB) and Center for Applied Research and Education in Microscopy (CAREM), University of Liege, Belgium.

Dorien VAN HEDE, Dental Biomaterial research Unit, University of Liege, Belgium.

France LAMBERT, DDS, MS, PhD. Professor and Head, Department of Periodontology and Oral Surgery, CHU of Liège, University of Liege, Belgium. Dental Biomaterial research Unit, University of Liege, Belgium.

Corresponding Author:

Deborah ROTH

Quai Godefroid Kurth, 45, B-4000 Liège, Belgium

deborah.roth@chuliege.be

ABSTRACT

Aim: To investigate the percentage of pollutants on customized abutments arrived from the implants company and the additional effect of dental laboratory manipulations. The second purpose was to validate the effectiveness of a simplified ultrasonic cleaning protocol to clear customized abutments before placement.

Materials and methods: Twenty-four customized abutments were divided in two groups of 12 titanium and 12 zirconia abutments returning from the implant company or the dental laboratory AND cleaned or not. The two steps cleaning protocol consisting of mechanical treatment with a sterile compress soaked in RBS T 105 (detergent) over the transgingival part of the abutment followed by 3 successive ultrasound baths for 2 min /bath. The presence of pollutants was quantified by scanning electron microscopy.

Results: The propose a cleaning method that allowed to significantly decreases the quantity of pollutants ($p=0.0006$). The abutments returning from the dental laboratory were significantly more polluted than those coming directly from the implant company ($p=0.0043$). The cleaning effect was highly significant in both groups ($p<0.0001$). The quantity of pollutants before cleaning were similar in the titanium and in the zirconia groups and the cleaning effect was highly significant in both groups ($p=0.0009$).

Conclusion: The tested cleaning protocol tested performed on the customized abutments whether they come back from the implant company or the dental laboratory.

Keywords: customized abutment, contamination, ultrasonic cleaning, disinfection, sterilization,

INTRODUCTION

The formation of an early and long-standing effective barrier to protect the peri-implant structures is essential to avoid bacterial penetration that could jeopardize the initial healing and long-term implant survival. This biological seal, known as the biological width, is composed of two types of tissular interface, coronally, an epithelial adhesion and below a connective adhesion [1–6].

Physico-chemical properties of the abutment seem to be a critical factor for the peri-implant soft tissue integration[1,7]. It was previously reported that abutment surface properties influence the adhesion, proliferation, and colonization of fibroblastic cells and microorganisms, and are considered the key influencing factors of a stable and healthy transmucosal seal[8].

However, the manufacturing process or the laboratory procedures may contaminate the transmucosal abutments and the residues may lead to an undesired biological response. It was already suggested by several authors that a proper cleaning of the abutment must be carried out before placement[9–12]. However, a survey study among 85 universities over the world, revealed that 25% of the centers do not apply any abutment cleaning procedure, 57% use steam vapor, ultrasonic bath or a disinfectant (Chlorhexidine, H_2O_2, Glutaraldehyde) and finally 18% use a combination of the preceding treatments[13]. The most common component cleaning methods proposed in the literature are those using argon plasma, steam or a series of ultrasonic (US) baths[10,14–16]. Canullo and coworkers compared the cleaning efficacy of Argon plasma versus a rather long (45 min) US protocol using successive baths of antibacterial detergent, pure acetone and ethylic alcohol. The results of this study suggest that both the argon plasma and US treatments can be equally adopted for abutment cleaning process[16]. More recently, other authors also emphasized that abutments cleaned in 3 successive US baths (anti-bacterial, alcohol, water) displayed less pollutants compared to abutments cleaned with steam, ethanol (wiping or soaking) or with chlorhexidine (scrubbing)[17–19].However, *in vitro* studies demonstrated that titanium abutments cleaned with argon plasma allowed better fibroblastic and epithelial cell adhesion after cell seeding when

compared to unclean abutments[10,20]. Canullo and co-workers also showed in a randomized controlled trials the interest of cleaning titanium abutments with argon plasma to enhanced cell adhesion[10].

While the studies mentioned above provide valuable information on the cleaning method, a comparison of the amount of pollutants on the abutments returning from the implant company or from the dental laboratory has not yet been studied. The proposed US and argon plasma cleaning protocols seem to be the most effective, however, these are time-consuming and/or costly and may not always be realistic in daily practice. In the present study, we proposed a simplified US cleaning protocol consisting in rubbing with a compress soaked in detergent and then immersion in three successive US baths for two minutes each at room temperature (Detergent, Sterile Water, Ethanol). This method saves times and cost efficiency compared to the US techniques described in the literature.

The first objective of the present study was to investigate the efficacy of a simplified US cleaning protocol to decontaminate custom-made implant abutments. The null hypothesis states that the US cleaning protocol does not allow the removal of pollutants on the abutments. The second aim was to evaluate the degree of pollution of CAD-CAM titanium and zirconia abutments delivered by the company and the additional effect of laboratory manipulations.

MATERIALS ET METHODS

Study design

In this in vitro study, a total of 24 customized abutments made of different materials were analysed to quantify the amounts of surface pollutants by low vacuum scanning electron microscopy. The experimental cleaning procedure was applied to 12 abutments while the 12 others remained uncleaned. In each group, 6 abutments were directly delivered from the company while the 6 others were subsequently manipulated in the dental lab after making a provisional PMMA crown. The flowchart of the study design is represented in **(Figure 1).** One half of the abutments is in titanium (Ti) and the other half were in zirconia (ZrO_2) **(Figure 2a and b)**.

Specimen abutment preparation

The specimen abutments were produced by a CAD-CAM manufacturing process (Procera® Nobel Biocare) from a single custom-made abutment design made from a implant model with a conical connection implant (Nobel Active®, Nobel Biocare, Kloten, Switzerland) placed in position 11. A total of 12 Ti and 12 ZrO_2 abutments were manufactured. Half of each abutment set was sent to the dental lab in order to fabricate a CAD-CAM PMMA provisional crown. The process included polishing and finishing with a make-up directly on the model abutment.

The other experimental abutments did not receive any further handling and were kept in the delivering non-sterile package.

Abutment cleaning protocol

Half of the abutments was subjected to the experimental cleaning protocol based on 2 steps.

First, the transmucosal parts of the abutments were scrub with a sterile gauze previously soaked in RBS T®105 (detergent) and secondly, the abutments were treated by the sequence of 3 US baths (each for 2 min) in RBS T 105, in sterile water and finally in ethanol 70°, each for 2 min **(Figure 3a and b).**

Quantitative evaluation of the contamination

The amounts of pollutants on the 24 abutments were then assessed in a FEI XL-30 Filed emission gin-environmental scanning electron microscope (FEG-ESEM XL-30, FEI, Netherlands) working in low vacuum condition (0.4-0.6 Torr), at 15kV accelerating voltage and using the gaseous electron detector from backscaterred electrons (BSE, compositional images). The abutments were consistently positioned in the microscope chamber and a standardized region of interest (ROI) of 690 µm wide by 1293 µm long was defined at a magnification of 38 x **(Figure 4)**. Quantitative analyses of pollutants dots in the ROIs were carried out using a semi-automatic method (ImageJ® software, NIH, USA). The surface covered by pollutants in each ROI was then expressed in percent of the total area of the RIO.

Statistical analysis

Statistical tests were carried out using GraphPad 8.3.0. Normality tests (D'Agostino & Pearson) were performed following outliers' identification (ROUT test with Q = 1%) and withdrawal (n = 2). Unpaired t-test and Mann-Whitney test were performed to analyze the cleaning method effect and to compare the pollution coming from the Implant Company and the Dental Laboratory respectively. Two-Way ANOVAs were used to analyze the cleaning method effect in terms of origin (Implant company versus Dental laboratory) and composition (ZrO_2 versus Ti). Tukey's correction was applied for multiple comparisons. The data were presented as box and whiskers, associated to dots representing individual data.

RESULTS

The proposed US cleaning protocol allowed to significantly decrease the amount of pollutants (p=0.0006), with a pollution rate of 4.51 ± 3.41 % at baseline and dropping to 0.06 ± 0.05 % after applying the experimental cleaning procedure **(Figure 5a)**. Before applying the cleaning procedure, the abutments returning from the dental laboratory were significantly more polluted than those delivered directly from the

implant company (7.17 ± 2.56 % and 1.85 ± 1.41 %, p=0.0043), **(Figure 5b)**. The effectiveness of the experimental cleaning procedure was highly significant in abutments coming from both the company and the lab (p<0.0001) **(Figure 5c)**. Finally, looking at the influence of the abutment material (Ti versus ZrO_2) on the amount of pollutants before cleaning, no significant effect was found and the effect of the experimental cleaning procedure was highly significant with both materials (p=0.0009) **(Figure 5d)**.

DISCUSSION

The present study emphasized that custom-made CAD-CAM abutments delivered from a manufacturing center presented some surface pollutants and the laboratory procedures to fabricate a PMMA provisional crown increased significantly the quantity of surface pollutants. However, suggested the experimental cleaning protocol displayed a high efficacy to clean both the Ti and ZrO_2 abutments and the null hypothesis is therefore rejected.

Efficacy of the experimental cleaning protocol

Several abutment cleaning methods such as argon plasma, US bath or steam were described in the literature[10,14–19]. However most of them are time consuming and / or involve a certain cost. For example, some authors demonstrated the efficacy of a cleaning protocols using successive US warm bath of various products such as detergents and alcohol for up to 45 minutes[16,18]. Gehrke and co-workers also describe effective implant component cleaning method (Finevo Cleaning system, Bredent GmbH & Co. KG, Senden, Germany) using 3 successive US baths of 5 min consisting in antibacterial solution, ethanol and then pure water. On the monotype abutment, the proportion of surface covered by contaminants decreased from 4,86% (± 6,09%) to 0.19% (± 0, 13%)[17]. Although the protocol is half shorter and does not require warming of the bath(s), the results of the present study are equally effective as the surface

occupied by the contaminations decreased from 4.51±3.14% to 0.06 ± 0.05%. The cleaning protocol we proposed is indeed rather short and quite simple to implement in the dental office.

Other authors, also proposing a cleaning protocol, observed a decrease of component contamination density from 0.017% ± (0.016%) to 0.001% ± (0.001%)[19]. The low baseline level of contamination makes the comparison with the present study difficult, and it might be the consequence of pre-cleaning steps of the component with steam, ozone and UV disinfection. Additionally, most of the other studies did not measure the contamination baseline values of abutment coming directly from the milling center or returning from lab[11,16,18,19].

However, the authors observed that the steaming cleaning protocol is less effective when compared to the US or argon Plasma methods[18,19].

Although it involves a certain cost, the cleaning methods using argon plasma, also displayed higher cell adhesion in abutment retrieved in human when compared to uncleaned or steamed abutments. The authors concluded that beyond its abutment cleaning efficacy, Argon plasma treatment also favors peri-implant soft tissue healing and stability[10,20].

Effect of the dental laboratory procedure on the pollutant levels on abutments

In our study, we observed that the intervention of the dental lab on the abutments leads to a larger surface percentage coverd by pollutants (7,17±2,56%) in comparison with the abutments delivered from the implant company (1,85±1,41%). As previously suggested, the milling manufacturing procedures for customized implant abutments made of metals or zirconia may contaminate abutment surfaces through lubricants, waxes, generic pollutants and microparticles of metals(10,16). To our knowledge, the present study is the first one to emphasize the additional pollution of implant abutments returning from the lab after the realization of a provisional crown and without any precleaning procedure.

Indeed, most of the studies analyzed pollutants level after dental lab procedures including a precleaning step (with steam or other methods) or without a negative control(16–19) . However, Gehrke and co-

workers cited above showed that the hybrid abutments coming from de dental lab were less polluted (0,03±0,03%) than the monotype abutment manufactured in the milling center (4,86±6,09)(17). These results contrats with our findings, and the authors suggest that the polishing process of the hybrid abutment reduces the surface roughness which therefore may contribute to the abutment cleanness. However, these hybrid abutments bonded, polished, cleaned with ethanol in the lab were not subjected to the processing of a provisional crown and this may explain the contrast with our results.

Study limitations

The present study suffers from several limitations. It would have been relevant to investigate qualitatively the nature of the pollutants using energy dispersive elemental X-ray microanalysis (EDX). However, several authors observed that pollutants were both organic and inorganics compounds including carbon-rich deposits, silica-rich particles as well as other particles containing aluminium, sulfur and/or vanadium which may come from the CAD/CAM manufacturing and the dental lab manipulations[18,19]. Additionally, it should be clarified that abutments were not paired, different components were used for the test and control groups. Finally, it could have been relevant to also compare the proposed US cleaning protocol with a Steam protocol as it is often used in laboratory daily practice.

CONCLUSION

Within the limits of this study, the tested cleaning protocol on both customized titanium and zirconia abutments was effective, whether the abutments came back from the implant company or the dental laboratory. Despite the limited evidence on the clinical benefit of cleaning transgingival pieces, decontamination of these pieces prior to their placement may be reasonable to improve peri-implant tissue integration[10].

ACKNOWLEDGEMENTS

We would like to express our gratitude to Miljana Bacevic and the dental lab Bastin for their contribution.

REFERENCES

1. Abrahamsson I, Berglundh T, Glantz P-O, Lindhe J. The mucosal attachment at different abutments. An experimental study in dogs. J Clin Periodontol. 1998 Sep;25(9).
2. Abrahamsson L, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res. 1998 Apr;9(2):73–9.
3. Berglundh T, Lindhe J. Dimension of the periimplant mucosa. J Clin Periodontol. 1996 Oct;23(10):971–3.
4. Berglundh T, Lindhe J, Ericsson I, Marinello CP, Liljenberg B, Thornsen P. The soft tissue barrier at implants and teeth. Clin Oral Implants Res. 1991 Apr;2(2).
5. Buser D, Weber HP, Donath K, Fiorellini JP, Paquette DW, Williams RC. Soft Tissue Reactions to Non-Submerged Unloaded Titanium Implants in Beagle Dogs. J Periodontol. 1992 Mar;63(3):225–35.
6. Cochran DL, Hermann JS, Schenk RK, Higginbottom FL, Buser D. Biologic Width Around Titanium Implants. A Histometric Analysis of the Implanto-Gingival Junction Around Unloaded and Loaded Nonsubmerged Implants in the Canine Mandible. J Periodontol. 1997 Feb;68(2):186–97.
7. Welander M, Abrahamsson I, Berglundh T. The mucosal barrier at implant abutments of different materials. Clin Oral Implants Res. 2008 Jul;19(7).
8. van Brakel R, Meijer GJ, Verhoeven JW, Jansen J, de Putter C, Cune MS. Soft tissue response to zirconia and titanium implant abutments: an in vivo within-subject comparison. J Clin

Periodontol. 2012 Oct;39(10).

9. Teughels W, Van Assche N, Sliepen I, Quirynen M. Effect of material characteristics and/or surface topography on biofilm development. Clin Oral Implants Res. 2006 Oct;17(S2).
10. Canullo L, Penarrocha-Oltra D, Marchionni S, Bagan L, Penarrocha-Diago M, Micarelli C. Soft tissue cell adhesion to titanium abutments after different cleaning procedures: Preliminary results of a randomized clinical trial. Med Oral Patol Oral y Cir Bucal. 2014;
11. Gehrke P, Tabellion A, Fischer C. Microscopical and chemical surface characterization of CAD/CAM zircona abutments after different cleaning procedures. A qualitative analysis. J Adv Prosthodont. 2015;7(2).
12. Piattelli A, Farias Pontes AE, Degidi M, Iezzi G. Histologic studies on osseointegration: Soft tissues response to implant surfaces and components. A review. Dent Mater. 2011 Jan;27(1).
13. Canullo L, Tallarico M, Chu S, Peñarrocha D, Özcan M, Pesce P. Cleaning, Disinfection, and Sterilization Protocols Employed for Customized Implant Abutments: An International Survey of 100 Universities Worldwide. Int J Oral Maxillofac Implants. 2017 Jul;32(4).
14. Gehrke P, Smeets R, Gosau M, Friedrich RE, Madani E, Duddeck D, et al. The influence of an ultrasonic cleaning protocol for CAD/CAM abutment surfaces on cell viability and inflammatory response in vitro. In Vivo (Brooklyn). 2019 May 1;33(3):689–98.
15. Mehl C, Kern M, Zimmermann A, Harder S, Huth S, Selhuber-Unkel C. Impact of Cleaning Procedures on Adhesion of Living Cells to Three Abutment Materials. Int J Oral Maxillofac Implants. 2017 Sep;32(5).
16. Canullo L, Micarelli C, Lembo-Fazio L, Iannello G, Clementini M. Microscopical and microbiologic characterization of customized titanium abutments after different cleaning procedures. Clin Oral Implants Res. 2014 Mar;25(3).
17. Gehrke P, Abazari C, Schlichter K, Fischer C, Duddeck D, Romanos GE, et al. Qualitative and semi-quantitative assessment of processing-related surface contamination of oneand two-piece

CAD/CAM abutments before and after ultrasonic cleaning. Materials (Basel). 2020 Jul 1;13(14).

18. Kim S, Choi C, Cha Y, Chang J-S. The efficacy of convenient cleaning methods applicable for customized abutments: an in vitro study. BMC Oral Health. 2021 Dec 18;21(1).

19. Yin L, Zhu Y, Yu H, Qiu L. Evaluating the efficiency of three methods to clean and disinfect screw- and cement-retained prostheses. J Prosthet Dent. 2021 Jan;

20. Garcia B, Camacho F, Peñarrocha D, Tallarico M, Perez S, Canullo L. Influence of plasma cleaning procedure on the interaction between soft tissue and abutments: a randomized controlled histologic study. Clin Oral Implants Res. 2017 Oct;28(10).

TABLES AND FIGURES

---

1. Study design

2. Titanium (Ti)(a) and Zirconia ($ZrO_{2)}$(b) CAD/CAM abutments.

3. Sterile compress soaked in detergent (a) and Three times ultrasonic bath (b)

4. Low vacuum BSE- SEM image of the region of interest (RIO) for the quantitative analysis of pollutants.

5. Results a,b,c,d

6. Low vacuum BSE- SEM images (38x) of a titanium abutment returning from the implant company before (a) and after (b) cleaning protocol.

7. Low vacuum BSE- SEM images (38x) of a zirconia abutment returning from the implant company before (a) and after (b) cleaning protocol.

8. Low vacuum BSE- SEM images (38x) of a titanium abutment returning from the dental laboratory before (a) and after (b) cleaning protocol.

9. Low vacuum BSE- SEM images (38x) of a zirconia abutment returning from the dental laboratory before (a) and after (b) cleaning protocol.